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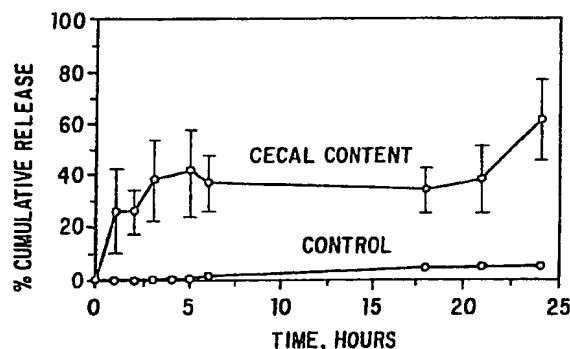
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(21) International Application Number: PCT/US91/03014 (22) International Filing Date: 2 May 1991 (02.05.91) (30) Priority data: 518,714 4 May 1990 (04.05.90) US (71) Applicants: YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM [US/US]; 11 East 69th Street, New York, NY 10021 (US). PERIO PRODUCTS LTD. [IL/IL]; 7, HaMarpeh Street, Fifth Floor, Har HaHotzvim, 91 999 Jerusalem (IL). (72) Inventors: SINTOV, Amnon ; 307/9, Achlama St., Gilo, 93 383 Jerusalem (IL). RUBINSTEIN, Abraham ; 24, Mevo Nahagei Hapredot, French Hill, 97 890 Jerusalem (IL).		(74) Agents: FOX, Samuel, L. et al.; Sterne, Kessler, Goldstein & Fox, 1225 Connecticut Avenue, N.W., Suite 300, Washington, DC 20036 (US). (81) Designated States: AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH (European patent), CI (OAPI patent), CM (OAPI patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GA (OAPI patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL (European patent), NO, PL, RO, SD, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent). Published With international search report.

(54) Title: COLONIC DRUG DELIVERY SYSTEM



(57) Abstract

A colonic delivery system for delivering a drug to the colon is provided. The system comprises a drug in combination with a matrix, wherein the matrix comprises a saccharide-containing polymer. According to the invention, the matrix is resistant to chemical and enzymatic degradation in the stomach and small intestine. The matrix is degraded in the colon by bacterial enzymatic action, and the drug is released. The system is useful for targeting drugs to the colon in order to treat colonic disease. The system is also useful for enteric administration of drugs such as proteins and peptides which are otherwise degraded in the stomach and small intestine.

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Title of the Invention**COLONIC DRUG DELIVERY SYSTEM**

This application is a continuation-in-part application of Application Serial No. 07/518,714, filed May 4, 1990.

5 Field of the Invention

The invention is directed to a drug delivery system for the delivery of enterally administered pharmaceuticals to the large bowel.

BACKGROUND OF THE INVENTION

10 Specific delivery of drugs and pharmaceutical compositions to the colon is important in the treatment of a wide variety of diseases and conditions. Targeting of drugs to the colon provides the ability to locally treat large bowel diseases, thus avoiding systemic effects of drugs or inconvenient and painful transcolonic administration of drugs. Furthermore, there is an increased need for delivery to the colon of drugs that are reported to be
15 absorbable in the colon, such as steroids, which would increase the efficiency and enable to reduce the required effective dose (Dogbillon, J., *et al.*, *Br. J. Clin. Pharmacol.* 19:113S (1985); Antonin, K.H. *et al.*, *Br. J. Clin. Pharmacol.* 19:137S (1985); Fara, J.W., *3rd International Conference on Drug Absorption*, Edinburgh (1988); for a review see Rubinstein, A.,
20 *Biopharm. Drug Dispos.* 11:465- 475 (1990)).

However, the targeting of drugs to desired locations in the alimentary canal can be complicated. Because of its location at the distal portion of the alimentary canal, the colon is particularly difficult to access. The design of orally administered colonic delivery systems must take into account factors
25 such as the pH of the alimentary canal and the presence of enzymes in the stomach and small intestine.

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In current techniques for targeting drugs to the colon, solid formulations of the desired drug molecules are coated with a pH resistant polymeric coating. Such formulations are similar to enteric coated formulations which may be used to deliver drugs to the distal ileum. Enteric coatings include bioerodable polymers such as shellac and cellulose acetate phthalate. (Levine *et al.*, *Gastroenterology* 92:1037-1044 (1987)).

In contrast to the enteric coated formulations, however, the formulations for colonic delivery are designed to withstand both low and slightly basic pH values (around seven) for several hours. During this time, they are assumed to pass the stomach and the small intestine and reach the large intestine, where the coat disintegrates and the drug release process is initiated. In this way, drugs such as 5-amino salicylic acid (5-ASA), and some steroids have been delivered to the colon.

The polymers used for this purpose are commonly acrylic acid derivatives or cellulose derivatives such as cellulose acetate phthalate, or ethyl cellulose (Rasmussen, S.N., *et al.*, *Gastroenterology* 83:1062 (1982); Levine, D.S., *et al.*, *Gastroenterology* 92:1037 (1987)); Mardini H., *et al.*, *Gut* 28:1084-1089 (1987). However, an important limitation of this technique is the uncertainty of the location and environment in which the coat starts to degrade. Depending upon the gastrointestinal motility pattern, which can vary widely in individual patients and in different disease states, degradation of the coating can occur deep in the colon, or within the small intestine.

The presence of short chain fatty acids, carbon dioxide, and other fermentation products, and residues of bile acids, often reduce the pH of the colon to approximately six (Stevens, C.E., *Amer. J. Clin. Nutr.* 31:S161 (1978); McNeil, N.I., *et al.*, *Gut* 28:707 (1987)). This change in pH calls into question the reliance on higher colonic pH as a trigger. U.S. Patent No. 4,627,850 (Ditter *et al.*) discloses an osmotic capsule for the controlled rate delivery of a drug comprising outer and inner walls each formed of a different polymeric material, the inner wall defining a space containing the drug, with a passageway through the walls connecting the exterior of the outer wall with the interior of the inner wall. U.S. Patent No. 4,904,474 (Theeuwes *et al.*)

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discloses a colonic drug delivery device comprising means for delaying the delivery in the drug and in the small intestine and means for delivering the drug in the colon. This device comprises osmotic means for forcing the active pharmaceutical agent out from the compartment in which it is contained through an exit provided in said compartment, into the colon. The means for delaying delivery in the stomach or in the small intestine are practically pH resistant coatings. The delay in delivery of the drug is time based, the structure is so calculated that the contents of the inner drug-filled space are not forced out before the device has reached the preselected target region of the gastro-intestinal tract. One of the drawbacks of these devices is that in case the travel of the device in the GI tract is delayed in a certain portion of the tract, for example due to mechanical reasons, the drug will still be released after the predetermined time has passed, irrespective of the fact that the target region has not been reached.

The ability of the colonic flora to degrade substrates that are resistant to small bowel digestion has been studied as an alternative method for colonic delivery of drugs. This principle was utilized to deliver laxative products, mainly sennoside and related compounds. Concentrated senna extract contains anthracene derivatives which exist in the form of glycosides and can be hydrolyzed to anthroquinones, anthranols, and oxanthrones. The sennosides are much more effective as laxatives when administered intact, compared to the sugar-free aglycones, probably because the sugar moiety provides protection against chemical breakdown in the small intestine (Fairbairn, J.W., *J. Pharm. Pharmacol.* 1:683 (1949)). Hardcastle and Wilkins demonstrated that when sennosides were directly administered to the colon, no laxative activity was observed as compared to administration of the same compounds previously incubated with feces or *E. coli*. It was postulated that bacteria liberate the free anthraquinones, which then promote colonic peristalsis via a local effect on the mysenteric plexus (Hardcastle, J.D., *et al.*, *Gut* 11:1038 (1970); Cummings, J.H., *Gut* 15:758 (1974)).

Bacteria can also act on the phenolic laxative sulisatin, in which two of the phenols are esterified with sulfate. The lack of arylsulfatase activity in

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the small intestinal mucosa allows the drug to pass intact to the colon, where bacteria convert it to the active hydroxy and dihydroxy derivatives. This is in contrast to the acetate ester of the diphenylmethane derivative bisacodyl, which is readily cleaved by the esterases in the small intestine to an active metabolite, which in turn stimulates water and electrolyte secretion from the colonic mucosa and results in laxation (Cummings, J.H., *Gut* 15:758 (1974); Moreto, M., *et al.*, *Arzneim. Forsch./Drug Res.* 29:1561 (1979); Gullikson, G.W., *et al.*, in *Pharmacology of Intestinal Permeation II*, Csaky, T.Z. (ed.), Springer-Verlag, Heidelberg, p. 419 (1984)).

Simpkins and co-workers recently compared the ability of the narcotic antagonists naloxone and nalmefene with their glucuronide conjugates to induce diarrhea in morphine-dependent rats. (In these animals the brain is sensitive to narcotic antagonists, and the animals are useful in the bioassay of the systemic delivery of narcotic antagonists intended for local colonic release.) Oral administration of the two drugs caused diarrhea, withdrawal behavior and tail skin temperature response within 15 minutes, while with the glucuronide conjugates of either of the narcotic antagonists diarrhea was delayed for 1 to 3 hours, reflecting the transit time to the distal ileum. Direct colonic administration of the naloxone and nalmefene glucuronides caused diarrhea with 5-8 minutes. It was suggested that the pharmacologic response to the glucuronide conjugates of naloxone and nalmefene was initiated by bacterial β -glucuronidases in the rat colon. This hydrolysis of the drug glucuronides was found to be specific to bacterial activity in the colon, because the glucuronides were inactive when administered subcutaneously (Simpkins, J.W., *et al.*, *J. Pharmacol. Exp. Ther.* 244:195 (1988)).

A drug traditionally used in the treatment of inflammatory bowel disease is sulfasalazine. Sulfasalazine is composed of the antibacterial sulfapyridine linked to the anti-inflammatory 5-ASA with an azo bond. When the drug was first introduced in 1941, the sulfa moiety was regarded as the major therapeutic determinant in the action of sulfasalazine. It was later recognized that the 5-ASA is responsible for the clinical effect, while the sulfapyridine causes most of the side effects of the drug (Khan, A.K., *et al.*,

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Lancet 2:892 (1977)). In fact, the sulfasalazine is a prodrug which carries the active 5-ASA to the colon, where bacterial azo reduction releases the molecule with the desired therapeutic properties (Klotz, U., *Clin. Pharmacokin* 10:285 (1985)).

5 Based on the understanding of the mode of action of sulfasalazine, a second generation of the sulfasalazine has been developed: azodisalicylate and salicylazobenzoic acid. Azodisalicylate is composed of two 5-ASA molecules in which the amino groups of the two molecules are linked through an azo group. When it is reduced by colonic bacteria, the azodisalicylate delivers
10 twice the amount of 5-ASA, and avoids the undesired action of the sulfapyridine (Willoughby, C.P., *et al.*, *Gut* 23:1081 (1982); Bartalsky, A., *Lancet* 1:960 (1982)).

 The 5-ASA prodrugs, including sulfasalazine, azodisalicylate and salicylazobenzoic acid, represent a slightly different approach from the classic
15 prodrug delivery concept in that release of the parent drug is mediated by bacterial enzymes located at the target organ, rather than by enzymes of the target tissues. The realization that enzymes characteristic of inhabitant microorganisms of the colon may convert prodrugs and other molecules to active therapeutics led to an increase in research activity in the area of
20 microbially controlled drug delivery to the colon.

 A modified method to deliver 5-ASA to the colon was reported by Brown, Parkinson and co-workers who, in order to eliminate the effects of the sulfapyridine fraction, azo-linked sulfasalazine to a high molecular weight
25 polymeric backbone. The resulting water-soluble polymer was shown to release 5-ASA in the presence of anaerobic rat cecal bacterial.

 Pharmacokinetic analysis of 5-ASA levels following the polymeric prodrug administration showed similar deliveries of 5-ASA and metabolites to the lower bowel, blood, and urine of orally dosed rats. Pharmacodynamic
30 analysis showed that the polymer also decreased the carrageenan-induced ulcerative-colitis-like inflammatory response in guinea pigs, based on quantitative histopathological results. This pharmacodynamic response was

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found to be equal to the one achieved after direct administration of 5-ASA and superior to sulfasalazine (Brown, J.P., *et al.*, *J. Med. Chem.* 26:1300 (1983)).

5 Friend and Chang glycosylated selected steroid drugs commonly used in the treatment of inflammatory bowel disease (hydrocortisone, prednisolone, dexamethasone, and fluorocortisone). Glycosylation was accomplished using galactose, glucose, and cellobiose which are known to serve as substrates for colonic bacteria (Cummings, J.H., *et al.*, *Amer. J. Clin. Nutr.* 45:1243 (1987)). The glycoside prodrugs were incubated with homogenates of the contents of various regions of the rat alimentary canal. In the stomach, 10 proximal ileum, and distal ileum, it was found that the rate of hydrolysis of all prodrugs was relatively slow. However, the rate of hydrolysis was higher in homogenates of the contents of the cecum.

The authors concluded that delivery of glycoside prodrugs to the colon depends upon the different rates of hydrolysis in the various segments of the 15 alimentary canal, the different transit times in those segments, and the octanol/water partition coefficient of the prodrugs. Thus, the faster transit in the upper GI tract coupled with its slow observed degradation activity, and the slow transit in the cecum, in which relatively faster degradation occurs, suggest the potential use of glycoside prodrugs to treat large bowel disease (Friend, D.R., *et al.*, *J. Med. Chem.* 28:51 (1985)). 20

The covalent functionality of azoaromatic compounds, susceptible to cleavage by the colonic bacteria, was recently utilized by Saffran and co-workers (Saffran, M., *et al.*, *Science* 233:1081 (1986); Saffran, M., *et al.*, *J. Pharm. Sci.* 77:33 (1988)). Assuming that the distal part of the small 25 bowel and the colon are the preferred sites for intestinal absorption of protein drugs, insulin and lysine-vasopressin solid dosage forms (pellets and gelatin minicapsules) were coated with copolymers of styrene and hydroxyethylmethacrylate cross-linked with divinylazobenzene.

It was postulated that this polymer is able to protect the entrapped 30 protein drugs against the digestive enzymes of the stomach and the upper portion of the small intestine, and that the polymer is degraded upon arrival at the colon. Indeed, when incubated in fecal content of rat or human for

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eight days, perforation of the polymer coat was microscopically detected. In addition, sustained pharmacological response of the protein drugs, antidiuresis for lysine-vasopressin, and hypoglycemia for insulin, was observed when the coated delivery systems were orally administered to rats, and later to dogs (Saffran, M., *et al.*, *Diabetes* 38S:81A (1989)).

A delivery system of antiamoebic drug, relying on specific phagocytosis of the carrier by *Entamoeba histolytica*, which is confined to the lumen of the large intestine, has been reported (Mirelman, D., *et al.*, *J. Infect. Dis.* 159(2):303 (1989)). Small silica particles covalently linked to nitroimidazole-based drug were designed to eliminate the parasite from the lumen of diseased humans. It was found that *E. histolytica* trophozoites avidly phagocytosed the tiny particles and released the bound drug, causing rapid cell death of the trophozoites both *in vitro*, and *in vivo* in hamsters. Although the amount of digested particles did not exceed 5% of the total number of particles, it was stated that this was enough to cause the death of most of the trophozoites population in 24 hours. In the absence of amoebic trophozoites, no significant release of the covalently bound drug was observed.

While there is evidence that certain proteins and peptides such as interleukin-II, interferon, colony-stimulating factor, tumor necrosis factor, and melanocyte-stimulating hormone may create new and effective therapies for diseases that are now poorly controlled, the acceptance of these proteins as drugs is currently limited by the methods of delivery. Colonic delivery may be a preferred route of administration for these and other new protein and peptide drugs.

Colonic delivery is also important for targeting drugs to the colon, particularly for the treatment of inflammatory bowel disease (IBD) and ulcerative colitis. However, the currently available enterally administered preparations of drugs designed for colonic delivery are not feasible for long-term use in humans, in part because of the potential toxicity of the azo compounds. There exists a need for an improved colonic delivery system that can be used with a wide variety of drugs and bioactive compounds.

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SUMMARY OF THE INVENTION

The invention is directed to a colonic delivery system comprising a drug, in combination with a matrix, such matrix comprising a saccharide-containing polymer, and such matrix being resistant to chemical and enzymatic degradation in the stomach and small intestine of the subject who is administered the colonic delivery system.

The colonic delivery system of the invention further provides a method for enterally administering a drug or other bioactive compound to a patient in need of such drug whenever it is necessary that such drug escape gastrointestinal digestion.

The colonic delivery system of the invention further provides a method for delivering efficacious levels of drugs designed for the treatment of diseases of the colon to the colon.

The invention further provides methods for the preparation of oligosaccharide-containing polymers, suitable as matrices for the drug delivery systems of the invention. More particularly, the invention provides methods of modifying natural polymers, such as mucopolysaccharides and particularly chondroitin and pectin, to be suitable as matrices for the drug delivery system of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Typical U.V. spectrum of chondroitin and modified chondroitin products in hydroalcoholic solutions. 1: chondroitin, 2: RMN 70; 3: RMN 60; 4; RMN 55.

Figure 2: Summary of "short term" (5 hours) experiments: cumulative amount of indomethacin released from three formulations, RMN 70, RMN 60 and RMN 55, in various dissolution media: (○) PBS (control), (○) rat cecal content in PBS, (Δ) sonicated rat cecal content in PBS.

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Figure 3: Cumulative percentage of indomethacin released from RMN 70 formulation as analyzed in rat cecal content medium (○) and in PBS (○). The data are the mean of 3 sets of experiments.

Figure 4: Cumulative percentage of indomethacin released from RMN 60 formulation as analyzed in rat cecal content medium (○) and in PBS (○). The data are the mean of 3 sets of experiments.

Figure 5: Cumulative percentage of indomethacin released from RMN 55 formulation as analyzed in rat cecal content medium (○) and in PBS (○). The data are the mean of 3 sets of experiments.

Figure 6: Total indomethacin released after 28 hours from the three chondroitin formulations in rat cecal content and PBS control. The data are the mean of 3 different sets of experiments.

Figure 7: Cumulative release of indomethacin from pectic salt matrix with and without pectinolytic enzyme.

Figure 8: Cumulative release of indomethacin from pectic salt matrices in the presence of rat cecal content as compared to its release in PBS (control) dissolution medium.

Figure 9: Plasma levels of indomethacin after intra-intestinal administration of modified chondroitin colonic delivery system and hydroalcoholic dispersion in dog.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions

In the description that follows, a number of terms used in pharmacology are extensively utilized. In order to provide a clear and consistent understanding of the specification and claims, and the scope to be given such terms, the following definitions are provided.

By the term "colon" is meant that part of the large intestine that extends from the cecum to the rectum. The cecum is the blind pouch in which the large intestine begins and into which the ileum opens from one side.

By the term "matrix" is meant a material comprising a saccharide-containing polymer, wherein the saccharide-containing polymer is preferentially degradable in the colon.

By being "preferentially degradable in the colon" is meant that the substance, when taken orally by a subject, (1) is relatively resistant to chemical and enzymatic degradation in the stomach and small intestine of such subject, and (2) is relatively susceptible to degradation in the colon so as to be capable of providing or otherwise releasing efficacious levels of a desired drug(s) in the colon of such subject.

By the term "saccharide-containing polymer" is intended polymeric constructs comprising a synthetic oligosaccharide-containing biopolymer or saccharide-containing natural polymers. Examples of synthetic oligosaccharide-containing polymers useful in the compositions and methods of the invention include methacrylic polymers covalently coupled to oligosaccharides such as cellobiose, lactulose, raffinose, and stachyose. Examples of saccharide-containing natural polymers useful in the methods of the invention include modified mucopolysaccharides such as cross-linked chondroitin sulfate and metal pectin salts, for example calcium pectate.

By the term "drug" is meant any pharmaceutical or physiological agent, composition, bioactive compound, or combinations thereof, useful in the diagnosis, cure, mitigation, treatment, or prevention of a disease, or for

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any other medical purpose. The term "drug" is intended to be interpreted broadly and is not limited in terms of chemical composition or biological activity.

5 The invention is directed to a colonic delivery system comprising a drug in combination with a matrix, said matrix comprising a saccharide-containing polymer. The colonic delivery system of the invention is based upon the ability of the colonic bacteria to digest substances that are not degraded in the stomach or the small intestine, or are degraded to only a minor extent.

10 The colonic delivery system of the invention serves as a means to target enterally administered drugs to the large intestine. When the drug-matrix composition of the invention is present in the stomach or the small intestine, its drug content is shielded by the matrix and is not affected by enzymes or pH of these organs. After the drug-matrix composition reaches the colon, bacterial enzymes degrade the matrix, thereby releasing the drug.

15 Accordingly, a subject in need of treatment with a desired drug, especially when it is desired to target the desired drug to the site of such subject's colon, may conveniently obtain such treatment by orally ingesting the composition of the invention. Alternatively, if desired, the composition of the invention may be provided in suppository form. Examples of drugs that may be provided in the composition, delivery system and methods of the invention include, for example, peptide and protein drugs such as analgesics, oral vaccines, plasminogen-activating peptides, contraceptive peptides, growth-promoting peptides, steroid drugs, such as dexamethasone, budesonide, beclomethasone, fluticasone, tiotropium and hydrocortisone, protein drugs that may survive longer and be absorbed better from the colon than from the small intestine, such as LH/RH and insulin, drugs which have been proved to possess colonic absorption such as theophylline, isosorbide dinitrate, nifedipine, oxprenolol, antispasmodic agents for the treatment of Irritable Bowel Syndrome such as cimetropium bromide, anti-neoplastic agents such as methotrexate, tamoxifen, cyclophosphamide, mercaptopurine and etoposide,

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other drugs such as cyclosporin, and monoclonal antibody-containing preparations. In addition, chemotherapeutic agents useful in the treatment or mitigation of colon tumors may be provided.

5 The therapeutic benefits of the colonic delivery system depend upon its ability to directly deliver efficacious levels of drugs to the colon. This allows the local treatment of colonic diseases, such as ulcerative colitis or colon carcinoma. Direct delivery of drugs to the colon enhances the amount of drug absorbed in the colon, and the amount of drug to which colon cells are directly exposed. Direct delivery or targeting of drugs also decreases the
10 systemic distribution of the drugs, thereby reducing undesirable and potentially harmful side effects. Additionally, some drugs are known to be more efficiently absorbed in the large intestine than in other portions of the GI tract. These include, for example steroids, xanthines and others. Direct delivery of such drugs to the large intestine would considerably decrease the required
15 effective dose.

In the controlled-release systems currently known in the art, drugs are released by diffusion mechanisms during transit of the drug-containing composition through the gastrointestinal tract. Once the drug reaches the lower portion of the gut, the release process became limited due to low fluid
20 content and high viscosity in this portion of the GI tract. This decrease in release rate leads to a decrease in drug absorption.

According to the present invention, however, these and other problems of current drug delivery methods are overcome by incorporating some of the drug into a suitable matrix (e.g., in a tablet core) which undergoes bacterial
25 degradation in the colonic surroundings and releases its drug content at least at efficacious levels, leading to improved bioavailability of the drug.

The flora typically found in the human gastrointestinal tract is summarized in Table 1. The flora may change depending upon the physiological condition of the person or animal being treated. Drug delivery

may be designed to specifically target a type of flora known to be in abundance in the patient.

Table 1

The Human Gastrointestinal Flora

		<u>Stomach</u>	<u>Jejunum</u>	<u>Ileum</u>	<u>Feces</u>
5	Total bacteria count	0-10 ³	0-10 ⁵	10 ³ -10 ⁷	10 ² -10 ¹²
	<u>Aerobic or Facultative</u>				
	<u>Anaerobic Bacteria</u>
10	Enterobacteria	0-10 ²	0-10 ³	10 ² -10 ⁶	10 ⁴ -10 ¹⁰
	Streptococci	0-10 ³	0-10 ⁴	10 ² -10 ⁶	10 ⁵ -10 ¹⁰
	Staphylococci	0-10 ²	0-10 ³	10 ² -10 ⁵	10 ⁴ -10 ⁷
	Lactobacilli	0-10 ³	0-10 ⁴	10 ² -10 ⁵	10 ⁶ -10 ¹⁰
	Fungi	0-10 ²	0-10 ²	10 ² -10 ³	10 ² -10 ⁶
	<u>Anaerobic bacteria</u>				
15	Bacteroides	Rare	0-10 ²	10 ³ -10 ⁷	10 ¹⁰ -10 ¹²
	Bifidobacteria	Rare	0-10 ³	10 ³ -10 ⁵	10 ⁸ -10 ¹²
	Gram-positive cocci ^a	Rare	0-10 ³	10 ² -10 ⁵	10 ⁸ -10 ¹¹
	Clostridia	Rare	Rare	10 ² -10 ⁴	10 ⁶ -10 ¹¹
	Eubacteria	Rare	Rare	Rare	10 ⁹ -10 ¹²

20 "Includes *Peptostreptococcus* and *Peptococcus*.
(from Simon, G.L., et al., *Gastroenterology* 86:174 (1984))

In one preferred embodiment, methacrylic polymers are employed because of their chemical stability in a biological environment. In particular, the polymers are not catabolized and absorbed in the gastrointestinal tract. These polymers do not contain extractable irritating compounds and have been demonstrated to be useful in, for example, surgical, ophthalmological, and dermatological applications.

The oligosaccharides that are covalently bonded to the acrylic polymers are preferably those which can be digested by colonic bacteria but not by the enzymes of the stomach or the small intestine. Examples of such

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oligosaccharides are cellobiose (4-O- β -D-glucopyranosyl-D-glucopyranose), lactulose (4-O- β -D-galactopyranosyl-D-fructo-uranose), the trisaccharide raffinose (α -D-Gal-[1 \rightarrow 6]- α -D-glc- β -D-fru), and stachyose (α -D-Gal- α -2-D-Gal- α -D-Glc- β -D-Fru).

5 Several methods of coupling the oligosaccharides to the acrylic monomer can be used, some of which are direct and others of which involve at least two steps.

10 In an example of a direct method, esters of methacrylic acid with sugar alcohols can be prepared by the trans-esterification of methyl methacrylate or by acylation with methacryloyl chloride.

15 Theoretically, numerous hydroxyl groups in oligosaccharides can react. However, the monosubstitution of the methacrylic acid is primarily in the 6 position (the primary alcohol). By using an excess of methacryloyl chloride or methyl methacrylate relative to the oligosaccharide used, a diester is produced which is useful as a cross-linking unit in the polymerization process.

20 In one example of a two-step method, a reactive group is introduced at a specific site on the oligosaccharide. A preferred embodiment, which does not require protection of the hydroxide groups, is reductive amination at the reducing end of the oligosaccharide, to form the acrylic 1-amino-1-deoxy-alditol. In the reductive amination step, ammonia may be used. Alternatively, a suitable diamine may be used. One amino group of the diamine binds to the sugar, leaving the second amino group available for the second step reaction with the acrylic monomer.

25 The amination can be performed using reactants such as ammonium bicarbonate, ammonium acetate, ethylene diamine or 2-(4-aminophenyl)-ethylamine. The reduction of the amino sugar can be performed with sodium borohydride, sodium cyanoborohydride, hydrogen gas with platinum oxide, paladium (10% Pd/C) or Raney Nickel.

30 After the amination of cellobiose, the glycosylamine produced is combined with methacryloyl chloride or with methacrylic acid to produce the methacrylic monomer.

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Polymerization is carried out to produce homopolymers of the previously synthesized monomer-oligosaccharides, or preferably to produce copolymers with monomers such as acrylate methacrylate, hydroxypropyl-methacrylate or hydroxyethyl-methacrylate.

5 Natural polymers such as mucopolysaccharides can also be degraded by colonic bacteria. The enzymes responsible for the bacterial catabolism of these polymers vary from polymer to polymer, and they can be either cell-associated or extra-cellular enzymes.

10 However, most of these natural polymers are, in their unmodified form, soluble in water and gastric fluid, and thus are not suitable as carriers for colonic drugs without modification. For example, chondroitin sulfate, which is a mucopolysaccharide, is a very soluble polymer and as a solid dosage form disintegrates quickly in water. Chondroitin sulfate has been reported as a substrate for the bacteroid inhabitants of the large intestine,
15 mainly *B. theta* and *B. ovatus* (Salysers, A.A., *Amer. J. Clin. Nutr.* 13:158-163 (1979); Salysers, A.A. and O'Brien, M., *J. Bacteriol.* 143:772-780 (1980)). It was suggested that periplasmic enzymes are responsible for the breakdown of chondroitin, probably by an outer membrane receptor which binds chondroitin sulfate and brings it into contact with enzymes such as
20 chondroitin-sulfate lyase.

Cross-linking methods may be used to reduce the hydrophilicity of these polymers and thus allow their utility in the compositions and methods of the invention as colonic drug carriers which pass the small intestine and degrade in the colon. An example of a preferred cross-linking method is
25 amide protection by the reaction of diamine with the polymer. Diamines that can be used include: 1,4 butanediamine, 1,6 hexanediamine, 1,7 heptanediamine and 1,12 dodecanediamine.

Thus, the invention provides a method of modifying chondroitin sulfate wherein chondroitin sulfate is brought into contact with a diamine
30 compound selected from the group consisting of 1,4-butanediamine, 1,6-hexanediamine, 1,7-heptanediamine and 1,12-dodecanediamine, in a suitable medium in the presence of a suitable catalyst, for a suitable period of time,

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the product is dialyzed in water and then lyophilized. 1,12-dodecanediamine is a preferred amine. Said medium is preferably dimethylsulfoxide or dimethylformamide. The catalyst is preferably dicyclohexylcarbodiimide.

5 The invention also provides a method of modifying pectin, wherein an aqueous solution of pectin is mixed with a metal chloride solution in which the concentration of the salt is adjusted to the desired degree of solubility of the final product using methods known in the art, the pH of the mixture is adjusted to 8-8.5 with sodium hydroxide to form a gel, followed by a precipitate, which is centrifuged and rinsed with water. The solid metal salt
10 of pectin is obtained, which is sieved to give a powder. Suitable metals salt of pectin include, for example, the calcium, strontium, and magnesium salt, with calcium being preferred.

After preparing the oligosaccharide-polymer matrix, the matrix is combined with a drug. Methods are known for formulating a composition to
15 allow controlled release of the chosen pharmaceutical compound. Using these and other known methods, compositions of the desired pharmaceutical compound may be formulated with the polymers of the present invention. Examples of such methods are disclosed in Saffran *et al.*, *Science* 233:1081-1084 (1986) and Levine *et al.*, *Gastroenterology* 92:1037-1044 (1987).

20 Specific embodiments of prepared formulations of the compositions of the invention, include, for example, matrix-drug tablets, especially tablets prepared by compression; matrix-drug pellets, either free or packed in gelatine capsules, or any other means allowing oral administration; matrix-drug nanoparticles, either free or packed in gelatine capsules or any other
25 means allowing oral administration; and multi-layered tablets which comprise cored drug, coated with biodegradable polymers, the polymeric layer being prepared, for example, by spray-coating, molding or double-press procedure. All of these techniques for preparation of such formulations are well known in the art.

30 The amount of drug can vary as desired for efficacious delivery of the desired drug and in consideration of the patient's age, sex, physical condition, disease, and other medical criteria. In addition, the amount of drug

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delivered by the system of the invention will depend upon the relative efficacy of the drug. The amount of specific drug necessary for efficacious results in the delivery system and methods of the invention may be determined according to techniques known in the art. For example, recommended dosages such as known in the art (*for example, see the Physicians' Desk Reference*, 1991 (E.R. Barnhart, publisher), *The Merck Index*, 10th Edition, Merck & Co., New Jersey, and *The Pharmacological Basis of Therapeutics*, 8th edition, A.G. Goodman *et al.*, eds., Pergamon Press, New York), provide a basis upon which to estimate the amount of a drug which has been previously been required to provide an efficacious level of activity. Especially, amounts of a desired drug that have previously been administered by suppository formulations, and the known characteristics of such drug when administered by suppository, are useful in this regard. Since the delivery system of the invention does not depend upon systemic (blood) delivery of the drug to the colon, it may be expected that efficacious levels of colon drugs that must be administered to a patient systemically will be higher than efficacious levels of such drugs when delivered directly to the colon.

Examples of drugs whose efficacious amounts for use in the delivery system of the invention may be determined in this manner include anti-inflammatory agents including non-steroidal and steroidal anti-inflammatory agents, dexamethasone, budesonide, beclomethasone, fluticasone, tiocortol, and hydrocortisone, cyclosporin, theophylline, nifedipine, isosorbide dinitrate, oxyprenolol, cimetropium bromide, anti-neoplastic agents including methotrexate, tamoxifen, cyclophosphamide, mercaptopurine etoposide, and indomethacin.

Tablets and capsules may be prepared and tested by techniques well known in the art, for example, as described in *Remington's Pharmaceutical Sciences*, Mack Publishing Company, 16th edition, 1980, and especially in chapter 89, the pharmaceutical preparation and manufacture of "Tablets, Capsules and Pills." In all embodiments, if desired, more than one drug may be supplied to the patient in the same matrix.

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In the tablet embodiments, for example, the compositions of the invention may provide a wide range of drug amounts, for example, the amount of drug can vary from about 5 to 30% by weight.

5 In another embodiment, a compressed tablet is formulated to contain efficacious levels of the desired drug(s) or pharmaceutical compound(s) as in the tablet embodiment, and an amount of the polymer of the invention that would allow disintegration of the tablet and release of the drug(s) following exposure of the tablet to one or more microorganisms present in the colon.

10 Other suitable embodiments will be known to those of skill in the art. A useful formulation will be suitable for enteric administration, will contain a drug targeted for release in the colon, and will further comprise an oligosaccharide-polymer matrix according to the invention. The formulation will be designed so as to allow protection of the drug from stomach and intestinal enzymes, but permitting degradation of the oligosaccharide-
15 containing matrix and release of the drug upon exposure of the formulation to colonic bacteria.

The delivery system and methods of the invention not limited to administration to humans and are especially useful for veterinary administration of drugs to any animal, including pets such as dogs, cats
20 horses, fish and birds, zoo animals, wild animal control and treatment, and agriculturally important animals of the food and dairy industry such as cattle, milk cows, swine and poultry.

25 The following examples further describe the materials and methods used in carrying out the invention. The examples are not intended to limit the invention in any manner.

EXAMPLE 1

Formation of Acrylic Oligosaccharide Monomers by One-Step Method

A. Trans-esterification

30 Two mmole raffinose was mixed with 6 mmole methyl methacrylate in 15 ml of dimethylformamide in the presence of 20 mg of 4-ethoxyphenol

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(MEHQ) and 10 mmole of sodium carbonate. The reaction mixture was heated to 70-75°C under vacuum of 100 mm Hg. A seven-plate fractionating column was installed for stripping the methanol from the system. After 12 hours the mixture was cooled. The product was identified using a TLC plate and further eluted from silica gel 60 column with ethylacetate.

B. Acylation

Two mmole raffinose was mixed with 6 mmole methacryloyl chloride, 10 mmole sodium carbonate (dried) and 20 mg of 4-ethoxyphenol in 15 ml dimethylformamide or dimethyl sulfoxide. The mixture was heated to 80°C for 7 hours under vacuum (100 mm Hg). Identification and purification were performed as in Example 1A.

EXAMPLE 2

Formation of Acrylic Oligosaccharide

Monomers by Two-Step Method

A. Three mmole of cellobiose and 3 mmole of sodium cyanoborohydride were mixed with 5 ml ethylenediamine (75 mmole) in a 25 ml flask at 5-10°C (ice bath). The reaction process was followed up using TLC plates and developing system of butanol-ethanol-water (5:3:2). The products were identified with iodine or ninhydrine spray.

B. 0.3 mmole of cellobiose was dissolved in 8 ml water, then 11 mmole sodium cyanoborohydride and 7.2 mmole ammonium acetate was added and mixed together in an ice bath. The same follow-up as in Example 1 was performed.

C. 3 mmole of cellobiose was dissolved in 33 ml of water, then 9 mmole of sodium cyanoborohydride was added and the mixture was cooled to 10°C in an ice bath. 60 mmole ammonium bicarbonate was added and the reaction was continued for 8 hours at 10°C and then for 64 hours at room

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temperature. Follow-up of the reaction process was performed by TLC as in Example 3, but with phenol sulfuric acid assay for identification. After 8 hours the mixture was evaporated to dryness, then 10 ml water was added and the mixture was again dried. Separation and isolation of the product was carried out in an Amberlite IR-120 (H) column (23 cm x 2 cm I.D.). The mixture (15 ml) was acidified to pH 5.5 with acetic acid, then 85 ml of water were added and the mixture was passed through the column (1.5 ml/min). The column was washed with 250 ml water then with 250 ml ammonia (0.7 M) and again with 250 ml water. The ammonia and the water fractions were collected and evaporated to dryness.

EXAMPLE 3

Linkage to Acrylic Monomer

A. Schotten-Baumann Reaction

Three mmole of the product of Example 2C was dissolved in 2 ml water and the solution was cooled in an ice bath to 2-4°C. Three mmole of NaOH was added to the solution. On cooling 3 mmole of methacryloyl chloride and an additional 3 mmole of NaOH in 2 ml of water were added dropwise and simultaneously. The reaction mixture was stirred at room temperature for an additional hour. Followup of the reaction was performed by TLC (silica gel plates, BuOH/EtOH/H₂O at a ratio of 5:3:2).

B. Three mmole of the product of Example 2C was stirred with 3 mmole of methacrylic acid in 5 ml dimethylsulfoxide. 3.3 mmole of dicyclohexylcarbodiimide (DCC) was added to the reaction mixture and this was stirred for 24 hours at room temperature.